

FIG. 1A

H36.D2.B7 Anti-Tissue Factor Light Chain Variable Region

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTACCATCACATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C
CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L
CTGATTTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTTCAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T
AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAATTATTACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y
AGTTCTCCATTACAGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

FIG. 1B

H36.D2.B7 Anti-Tissue Factor Heavy Chain Variable Region

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K
ACTTCTGGTTACTCATTCACTGACTACAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E
TGGATTGGATATATTGATCCTTACAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T
TTGACTGTTGACAAGTCTTCCACCACAGCCTTCATGCATCTCAACAGCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D D S A
GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGGGGCCAAGGCACCACTCTCACAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V
TCCTCA
S S

* CDR regions underlined.

0990566-12101

| Antibody | Apparent K_d , M^{-1} | Apparent K_d , M |
|------------|--|---|
| By ELISA | | |
| D2 | 5.2×10^9 | 1.9×10^{-10} |
| I47 | 6.5×10^9 | 1.5×10^{-10} |
| K73 | 9.8×10^9 | 1.0×10^{-10} |
| K80 | 2.3×10^9 | 4.3×10^{-10} |
| L102 | 2.5×10^9 | 4.0×10^{-10} |
| L133 | 1.7×10^9 | 5.9×10^{-10} |
| By BLACore | | |
| <u>H36</u> | <u>3.1×10^{10}</u> | <u>3.2×10^{-11}</u> |
| I43 | 2.3×10^9 | <u>4.3×10^{-10}</u> |
| I47 | 3.2×10^9 | <u>3.1×10^{-10}</u> |
| L133 | 4.6×10^9 | <u>2.2×10^{-10}</u> |
| M107 | 1.1×10^9 | <u>9.1×10^{-10}</u> |

FIG. 2

| Antibody Name | % Inhibition Antibody Preincubated with TF/VIIa |
|---------------|--|
| D1 | 0 |
| D1B | 1 |
| H31 | 4 |
| <u>H36</u> | <u>95</u> |
| I43 | 1 |
| J131 | 7 |
| K80 | 0 |
| K82 | 0 |
| K87 | 1 |
| L97B | 7 |
| L101 | 0 |
| L102 | 0 |
| L105 | 0 |
| L133 | 0 |
| M5 | 1 |
| M107 | 34 |

FIG. 3

0590586-4301

| Antibody Name | <u>% Inhibition</u> TF Preincubated with Antibody Prior to Addition of VIIa | <u>% Inhibition</u> TF Preincubated with VIIa Prior to Addition of Antibody |
|---------------|--|--|
| D1 | 15 | nd |
| D1B | 48 | 12.7 |
| H31 | 64 | 21 |
| H36 | 0 | 0 |
| I43 | 68 | 55 |
| J131 | 38 | 11 |
| K80 | 12 | nd |
| K82 | 0 | nd |
| K87 | 0 | nd |
| L96 | 0 | nd |
| L101 | 38 | 11 |
| L102 | 14 | nd |
| L105 | 4 | nd |
| L133 | 13 | nd |
| M5 | 0 | nd |
| M107 | 0 | nd |

FIG. 4

| [rhTF], nM | [H36.D2], nM | H36.D2/rhTF Molar Ratio | Clotting Time (seconds) | % Inhibition of rhTF Function |
|------------|--------------|----------------------------|----------------------------|----------------------------------|
| 0.0048 | 0 | 0 | 102.3 | 0 |
| | 1.61 | 335.4 | 114.3 | 31.3 |
| | 3.23 | 670.8 | 121.3 | 45.8 |
| 0.023 | 0 | 0 | 77.6 | 0 |
| | 1.61 | 70.0 | 85.3 | 52.2 |
| | 3.23 | 140.0 | 91.1 | 65.2 |
| | 6.45 | 280.4 | 99.6 | 73.9 |
| 0.092 | 0 | 0 | 49.3 | 0 |
| | 3.23 | 35.1 | 65.8 | 65.2 |
| | 6.45 | 70.1 | 88.5 | 90.2 |
| | 12.90 | 140.2 | 113.3 | 95.7 |
| 0.46 | 0 | 0 | 32.6 | 0 |
| | 6.45 | 14.0 | 52.7 | 82.4 |
| | 12.90 | 28.0 | 80.2 | 96.7 |
| | 32.30 | 70.2 | 117.9 | 99.3 |
| 2.30 | 0 | 0 | 23.9 | 0 |
| | 16.10 | 7.0 | 47.1 | 94.4 |
| | 32.30 | 14.0 | 95.2 | 99.7 |
| | 64.50 | 28.0 | 115.3 | 99.9 |
| 11.52 | 0 | 0 | 22.2 | 0 |
| | 16.10 | 1.4 | 30.2 | 93.4 |
| | 32.30 | 2.8 | 46.0 | 98.8 |
| | 64.50 | 5.6 | 87.6 | 99.9 |
| | 161.30 | 14.0 | 114.0 | 100.0 |

FIG. 5

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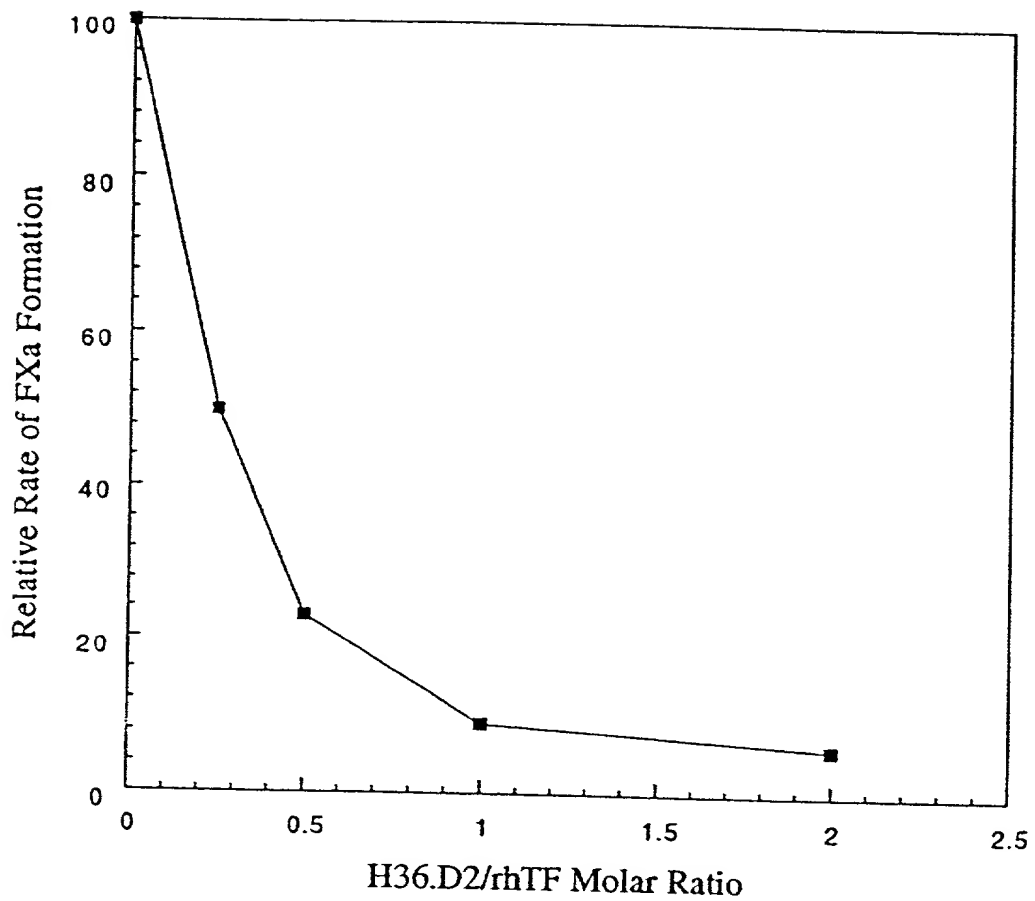


FIG. 6A

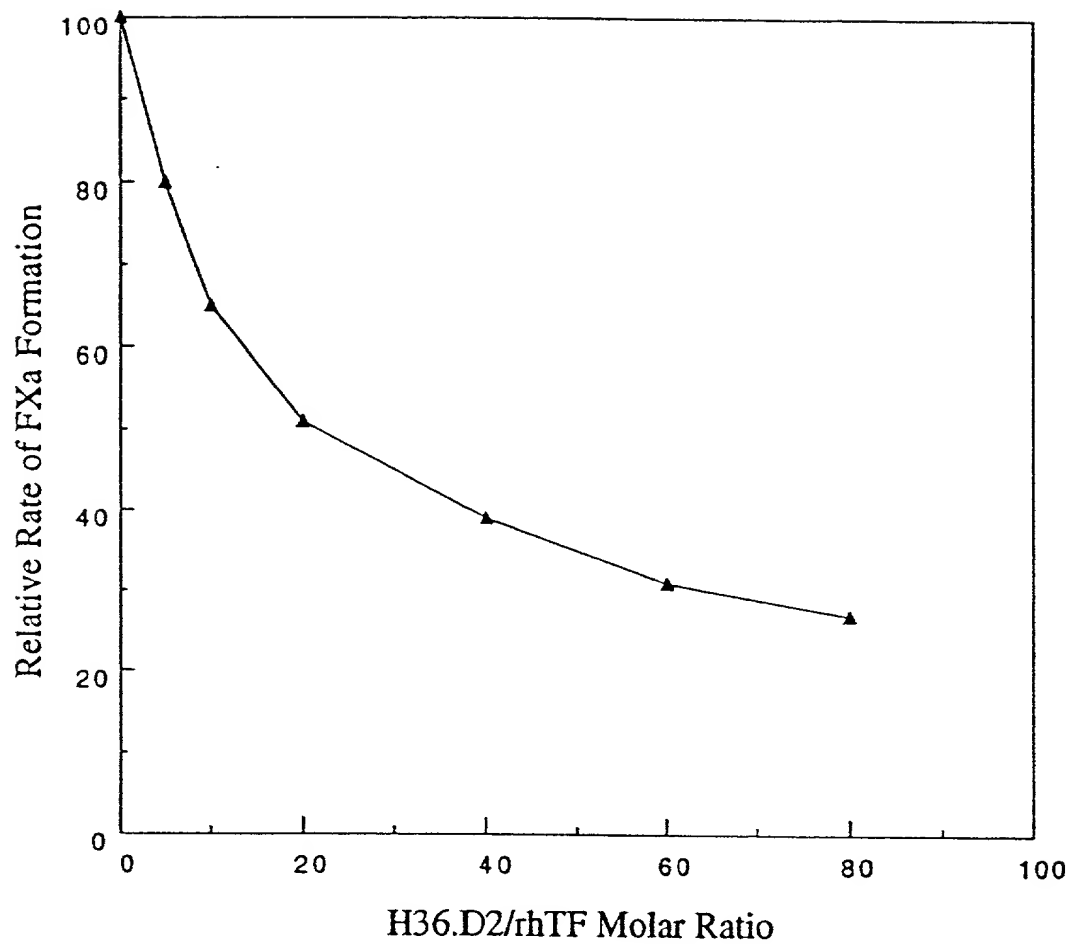


FIG. 6B

| H36.D2 Concentration (ng) | <u>% Inhibition</u> Cells (TF/FVII) and H36.D2 preincubated prior to FX addition | <u>% Inhibition</u> FX and H36.D2 are added simultaneously to Cells (TF/FVII) |
|------------------------------|---|--|
| 0 | 0 | 0 |
| 50 | 88 | nd |
| 100 | 92 | nd |
| 200 | 97 | nd |
| 800 | nd | 76 |
| 1600 | nd | 78 |
| 3200 | nd | 92 |

FIG. 7

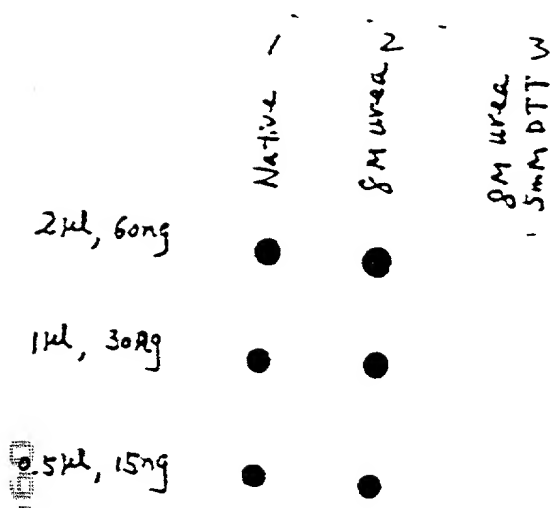


FIG. 8A

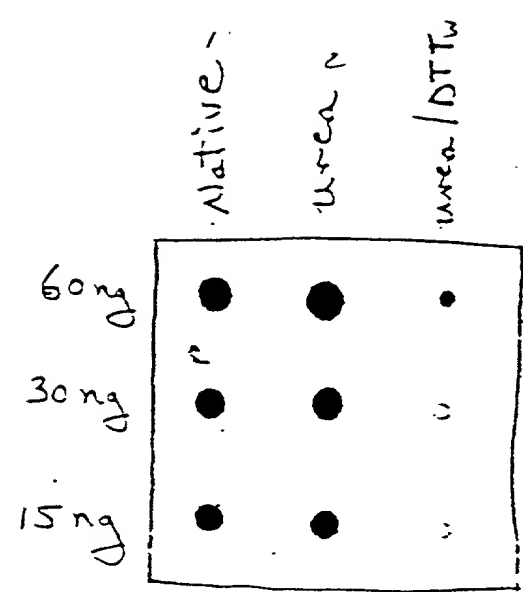
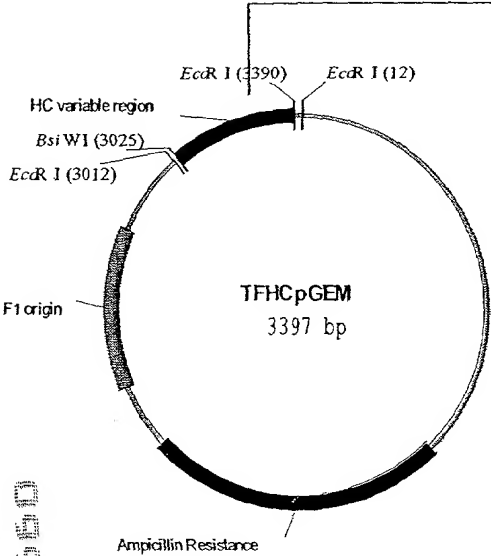


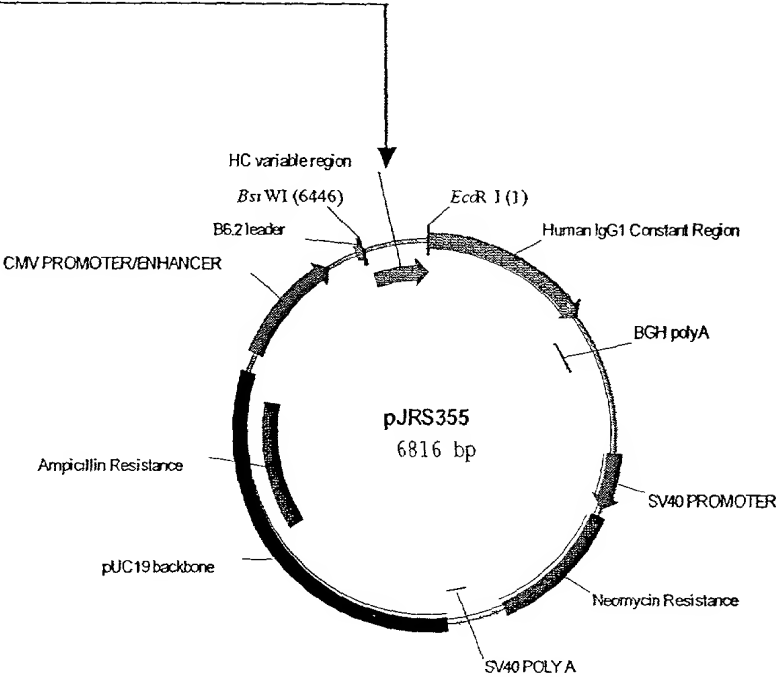
FIG. 8B

Figure A. Human IgG1-cH36 HC Variable Region Cloning and Expression Vector



HC Cloning Vector

Fig. 9A



HC Expression Vector

Fig. 9B

Figure B. Human IgG4-cH36 HC Variable Region Cloning and Expression Vector

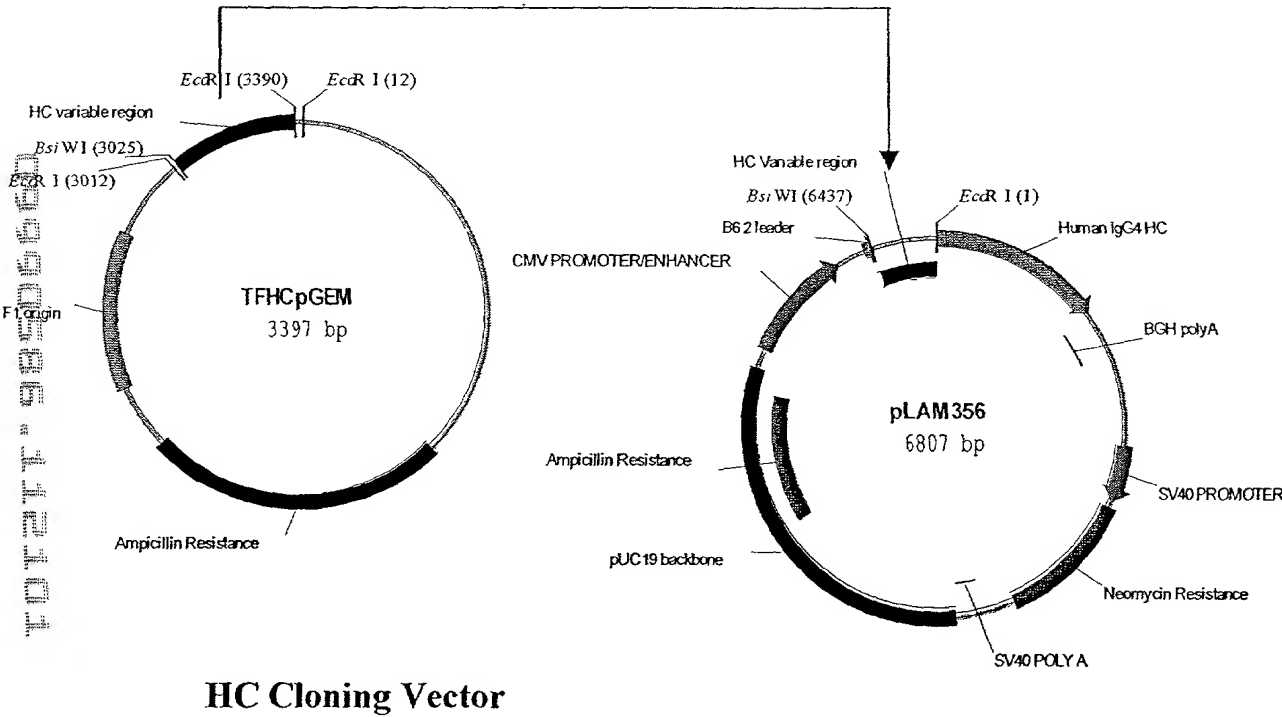


Fig. 9C

HC Expression Vector

Fig. 9D

Figure C. cH36 LC Variable Region Cloning and Expression Vector

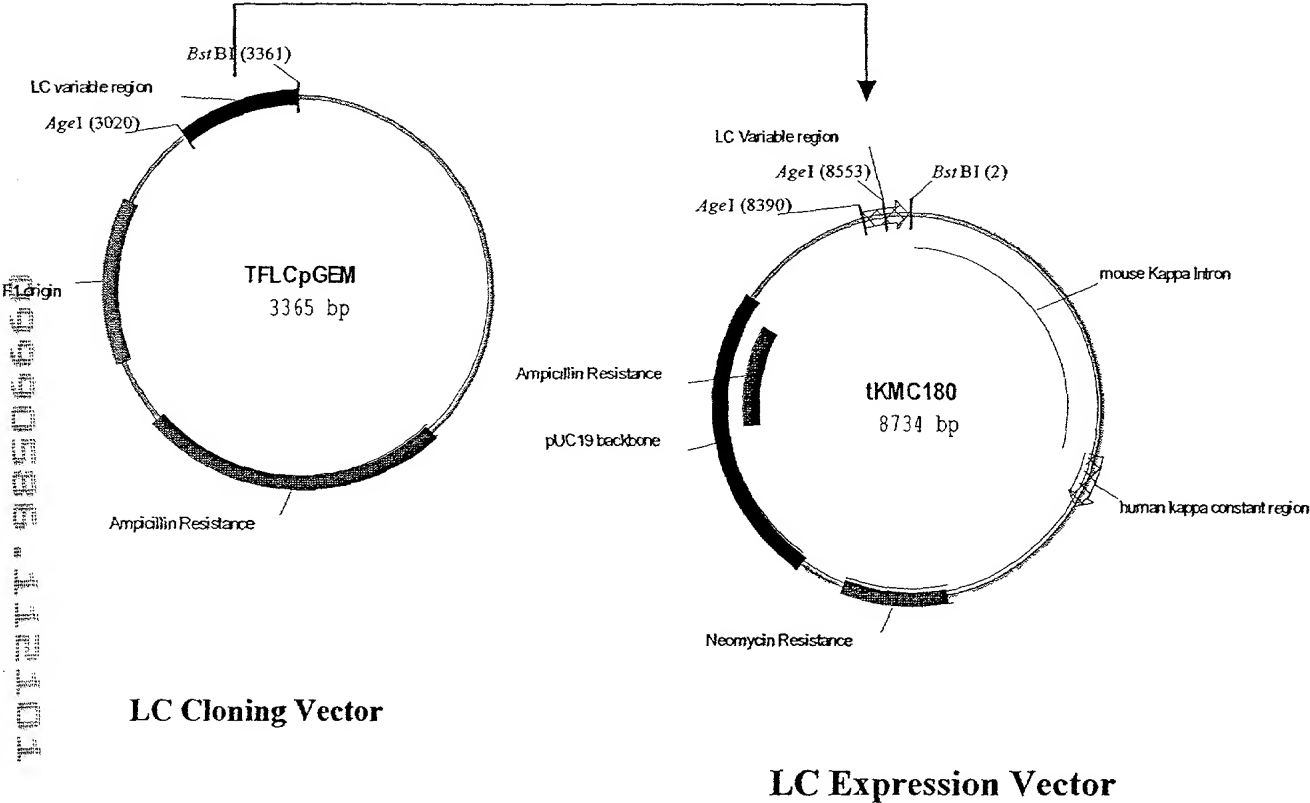


Fig. 10A

Fig. 10B

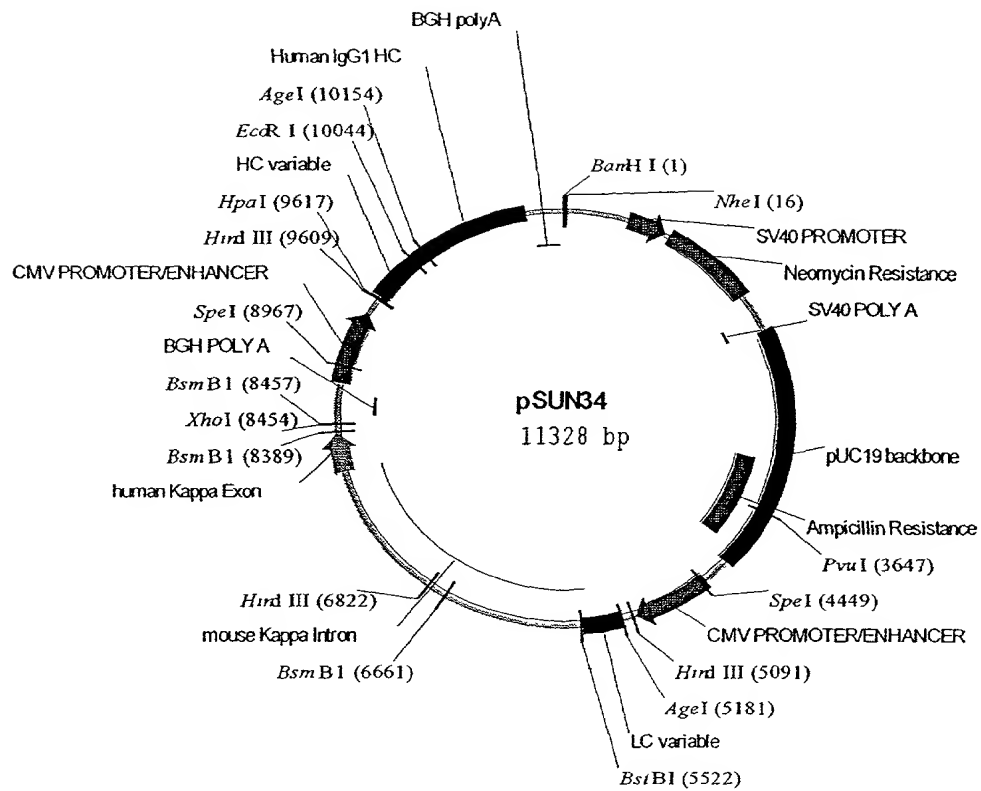


Figure D. Plasmid Map of Humanized Anti-TF IgG1 Antibody Expression Vector

Fig. 11

Humanization of anti-Tissue Factor Antibody cH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

Light Chain (LC) FR Sequences

| FR1 (23 AA) | FR2 (14 AA) | FR3 (32 AA) | FR4 (10 AA) | Names |
|---|-------------|-------------|-------------|---------|
| 1 10 20 35 47 57 60 70 80 86 98 107 | | | | |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | cH36-LC |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-03 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-04 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-05 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-06 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-07 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-08 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-09 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-10 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-11 |
| DIQMTQSPASQASLGE SV TI TC WYQQKPGKSPQLIY GVP SR FSGSGSGTK F SKISSLQAE DF VN Y YC FGAGTKLE L K | | | | LC-12 |

Fig. 12A

Light Chain CDR Sequences of cH36

| CDR1 (11 AA) | CDR2 (7 AA) | CDR3 (9 AA) |
|---|-------------|-------------|
| 24 34 50 56 89 97 | | |
| L A S Q T I D T W L A A A T N L A D Q Q V Y S S P F T | | |

Fig. 12B

Fig. 12C

Fig. 12D

Sequences of Partially and Fully Humanized Heavy Chain (LC) Variable Regions

Heavy Chain (HC) FR Sequences

| FR1 (30 AA) | FR2 (14 AA) | FR3 (32 AA) | FR4 (11 AA) | Names |
|-------------------------------|----------------|-----------------------------------|---------------|---------|
| 1 10 20 29 36 | 44 | 67 75 | 85 95 107 117 | |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSHGKSLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | CH36-HC |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSHGKSLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-01 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-02 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-03 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-04 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-05 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-06 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-07 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-08 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-08R1 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-11 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-12 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-09 |
| ELVQSGPELVKPGASVQVSCCKTSGYSFT | WVRQSGKGLLEWIG | KATLTVDKSSSTAFMHLNLSLTSDDSAVYFCAR | WGQGTTLTVSS | HC-10 |

1-6.
13A

Heavy Chain CDR Sequences

| CDR1 (5 AA) | CDR2 (17 AA) | CDR3 (8AA) | Names |
|-------------|-----------------------------------|-----------------|-------|
| 31 35 | 50 | 99 106 | |
| D Y N V Y | Y I D P Y N G I T I Y D Q N F K G | D V T T A L D F | CH36 |
| 31 35 | 50 | 99 106 | |
| D Y N V Y | Y I D P Y N G I T I Y D Q N F K G | D V T T A L D F | HC-08 |

Fig. 13C

Fig. 13D

hOAT (IgG1) Constant regions sequences

Sequences of LC constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC

Fig. 14A

Sequences of HC constant:

EFASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK

Fig. 14B

hFAT (IgG4) constant region sequences

Sequences of LC Constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC

Fig. 15A

Sequences of HC constant:

EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY
TCNVDPHKPSNTKVDKRVESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGV
EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYVTLPPSQEEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

Fig. 15B